

B. In the Specification:

Please amend the specification with a replacement substitute specification as shown in Appendix A as a "Clean Version." Appendix B includes a "Version with Markings to Show Changes Made." No new matter is presented in the replacement substitute specification.

C. In the Claims:

Please amend claims 1-16, as shown in Appendix A by a "Clean Version" and Appendix B by a "Version with Markings to Show Changes Made." The amended claims 1-16 contain no new matter. Applicants have amended certain claims in a sincere effort to advance prosecution of this Application. Applicants reserve the right to pursue the unclaimed subject matter in one or more continuing applications.

REMARKS

Applicants have addressed each issue in turn and, for clarity, have provided a heading for each issue.

Claim Election

Applicants appreciate Examiner's recognition of Applicants' election of Group I (claims 1-16), and SEQ ID NOS: 1-10, in Paper No. 7, filed June 8, 2001. Examiner has noted claims 1-76 are currently pending, but only claims 1-16 are under examination in this Action. Applicants believe no further response is necessary.

Sequence Rules Compliance

Applicants have amended sequence identifiers (specifically SEQ ID NO: 1-127811) into the specification at each sequence, as requested by Examiner. See Appendix A "Clean Version" and Appendix B "Version with Markings to Show Changes Made." Examiner requested an initial paper copy or compact disc copy of the "Sequence Listing." Applicants have provided two copies of the sequence listing on compact disc, labeled "Copy 1" and "Copy 2." Applicants have additionally included a computer readable form on compact disc, labeled "CRF."

Applicants have requested that a "REFERENCE TO SEQUENCE LISTING" heading and paragraph be inserted into the specification. Examiner has required a statement that the content of the sequence listing information recorded in computer readable form is identical to the written sequence listing. Applicants have included the statement in the amendment. The sequence listing and computer readable form contain no new matter.

Applicants' appreciate Examiner's reminder concerning the period for compliance with the sequence rules. Applicants believe this reply is fully responsive to this paragraph.

Priority

Applicants respectfully disagree with Examiner's assertion of alleged lack of grant of priority to the claimed Provisional Application, 60/100,678, filed September 17, 1998, due to lack of disclosure of the elected sequences in the instant Application. Applicants believe Examiner will appreciate priority between the two applications as the Applicants have provided disclosure of the elected sequences in the claimed Application. See Appendix A "Clean Version" and Appendix B "Version with Markings to Show Changes Made." Applicants are aware of no intervening prior art as disclosure of the claimed invention is contained in Provisional Application Serial No. 60/100,678, filed September 17, 1998. Applicants believe this reply is fully responsive to this paragraph.

Specification

A. Title

Applicants correct the title to "Methods of Genetic Analysis Using Nucleic Acid Arrays." See Appendix A "Clean Version" and Appendix B "Version with Markings to Show Changes Made." Applicants believe the corrected title to be descriptive. Applicants believe this reply is fully responsive to this paragraph.

Claim Rejections – 35 U.S.C. 101 and 112

Applicants respectfully traverse Examiner's rejection of claims 1-16 in light of 35 USC §101 and §112, 1st and 2nd paragraphs. Applicants assert that the claimed inventions are supported by credible assertions of specific and substantial utility within the specification. Additionally, Applicants assert that one skilled in the art would recognize specific and substantial utility as implied in the specification in the event that Examiner finds that an explicit assertion was not made by Applicants.

A. 35 USC §101

Applicants assert that the specification makes an explicit statement of specific and substantial utility.

On page 13, lines 1-18 of the Application as originally filed, Applicants provide examples of utility. Specifically, Applicants state:

...**hybridization patterns** can be compared to determine differential gene expression. As non-limiting examples: **hybridization patterns** from samples treated with certain types of drugs may be compared to **hybridization patterns** from samples which have not been treated ... **hybridization patterns** from [viral infected vs. non-viral infected samples] ... **hybridization patterns** for samples [with cancer vs. without cancer] ... **hybridization patterns** of samples from cancerous cells [treated with vs. not treated with tumor suppressing drugs].

as originally filed, page 13, lines 3-11 (emphasis added). Applicants have emphasized the phrase "hybridization patterns" to point out that the specific and substantial utility of the technology described in the specification is the use of the technology as a research tool. The technology can be used to compare samples by comparing the hybridization patterns developed by exposing those samples to the described technology.

Examiner focused on the descriptions of the claimed subject matter regarding gene expression analysis and stated that the critique in that area is generally applicable to all stated examples of uses for the claimed subject matter. Specifically, Examiner stated that after carrying out an experiment with the claimed subject matter, additional research was needed to find the significance of the results. Also, Examiner felt that the hybridization patterns from the experiments were generic to the use of any nucleic acid array.

Applicants respond by pointing out: 1) the results of an experiment utilizing the claimed subject matter can be a hybridization pattern that has real world significance to researchers, and 2) the nucleic acids within the array will generate a hybridization pattern that is unique and therefore, different from any other nucleic acid array generated from sequences not claimed in this Application.

- 1) **The claimed subject matter has substantial utility because the results of an experiment utilizing the claimed subject matter can be a hybridization pattern that has real world significance to researchers.**

A nucleic acid array is a tool that researchers can use in studying a wide variety of phenomenon. The claimed subject matter includes arrays made with specific nucleic acids. After experimentation the results generated will be hybridization patterns. The patterns in and of themselves have significance to a researcher. For example, a researcher studying differential gene expression after drug treatment would be interested in whether an untreated sample has a hybridization pattern different from the pattern of the treated sample. This alone may indicate an effect that the drug treatment has on the sample. The researcher would know that the drug does

something other than cause simple surface effects on physiology. The drug has an impact on gene expression levels (if the design of the hybridization was to look at mRNA levels within the samples). The researcher no longer has to guess whether the drug simply interacts with a surface molecule on the cell and nothing else, or if it has a more significant impact. The hybridization pattern would then be a "signature" of that drug's affect. This use is of real world significance to the researcher. It is not simply the production of an end result with no utility. The hybridization pattern is something with intrinsically useful information to a researcher.

The use of the claimed subject matter in a nucleic acid array is similar to the use of a specific column for high performance liquid chromatography (HPLC). When a researcher runs an HPLC experiment the results are most often presented as a graph of absorbance of light of a specific wavelength versus time of elution. The graph is simply a pattern of peaks and valleys. Upon visual inspection of the graph, a non-scientist or non-researcher, may conclude that the graph requires significant research in order to determine any significance. A researcher, however, is able to utilize that information adeptly to make conclusions about the analysis they performed utilizing the HPLC column. Likewise in the above example, a researcher can run the lysates of cells treated with a drug through the HPLC column. Also, the researcher can run lysates of untreated cells through the HPLC column. A comparison of the elution profiles of the two samples may be different. From that information alone the researcher knows that drug treatment causes a difference in the cellular constituents (in kind or in concentration). The elution profiles become signatures of the drug treated cells versus non-drug treated cells.

Like the HPLC column, an array made with the claimed subject matter of this Application provides a signature. The HPLC column is used to produce an elution profile while the array is used to produce a hybridization pattern. Both the elution profiles and the hybridization patterns can be used as signatures of drug treatment.

- 2) **The claimed subject matter has specific utility because the nucleic acids within the array will generate a hybridization pattern that is unique from any other nucleic acid array generated from sequences not claimed in this Application.**

Hybridization patterns from an array built from nucleic acids that are not part of the claimed subject matter cannot be the same. Even in the highly improbable event that the pattern of visualization of the hybridization pattern was fortuitously the same between an array with the claimed subject matter and an array built with other nucleic acid sequences, the information behind the patterns is unique. The sequences used to make an array impart specificity that cannot be duplicated by a generic DNA array. Additionally, if the researcher wishes to look behind the pattern and at the sequences that the samples hybridized to, the position of hybridization will allow the researcher to determine which sequences within the array were hybridized with the sample.

The use of the sequence information within the array is similar to using internal standards and controls via the HPLC column example from above. Once a researcher has performed HPLC analysis on drug treated versus untreated cell lysates, the elution profiles may be confusing because of experimental details. For example, the paper speed may have been different for each run. In that case the elution profiles by eye will have little relation to one another. Perhaps the researcher can discern that one profile is a compacted but similar to the other, but direct comparison would be difficult. A way to cure that problem is to put standards in the lysate that elute at known times and conditions so that the position of sample peaks in relation to the standard peaks can be compared. The standards can also be used as internal controls on the experiment. Perhaps the lysates include a substance that raises the baseline of absorbance so that the peaks seem too low to deserve concern. The standards can be put in the sample at a known amount so that the absorbance of the standard peak is known. From that information the baseline absorbance can be found by comparison to the intensity of the known standard peak.

Like the described HPLC experiment, arrays made with the claimed subject matter contain both standards and internal controls. The internal standards of the array are the sequences themselves. Arrays made with other sequences will not have the same standards. See, for example, on page 10, lines 9-18 of Application as originally filed. The mismatch sequences within the array act as internal controls. See, for example, page 10 lines 19-22 and page 11, lines 1-11 of Application as originally filed. Like in the HPLC experiment, the mismatch hybridization acts as a control in that the hybridization results may be deemed to be nonspecific if the hybridization of the experimental DNA is not more intense than the hybridization of the mismatch control. The sequences of the claimed subject matter, within an array impart tremendous specificity to the experiments.

Applicants appreciate Examiner's note that credibility has not yet been assessed. Applicants respectfully state that the assertion of utility would be considered credible by a person of ordinary skill in the art.

Applicants have provided statements in the specification, cited above, and arguments to support substantial utility for the claimed subject matter. Applicants also cite to U.S. Patent Nos. 5,800,992 (Fodor et al.) and 6,309,822 (Fodor, et al.) (on p.9 of Application as originally filed and p. 9 of Appendix A "Clean Version" of the Specification) and Zhang et al., Science 276 1268-1272 (p. 13 of Application as originally filed and p. 12 of Appendix A "Clean Version" of the Specification), all of which are directed toward research tools and methods. Applicants have further provided a publication regarding the commercial utility of the claimed invention. See Appendix C.

Applicants Response merely explains at least one specific and substantial utility. There are numerous alternative utilities that are not discussed in this Response. This Response is in no way intended to be limiting as to the utility of the claimed invention. Applicants believe this reply is fully responsive to this paragraph.

B. 35 USC §112, first and second paragraph.

Applicants respectfully traverse Examiner's rejection of claims 1-16 under 35 USC §112, first and second paragraph. Applicants' believe one skilled in the art would clearly know how to make and use the claimed invention as the Application has specific, substantial, and credible utility and in the alternative, a well-established utility as indicated in the above response. In addition, Applicants have amended the specification to clarify the terms: perfect match, perfect mismatch, antisense match and antisense mismatch. Applicant believes this clarifies the metes and bounds of these terms, as required by Examiner. Applicants believe this reply is fully responsive to this paragraph.

Objections (Warning)

Applicants appreciate Examiner's warning that should claim 1 be found allowable, claims 6-16 will be objected to under 37 CFR 1.75 as being substantial duplicates thereof. Applicants have requested amendment of claims 6-16 such that these claims are directed to methods of use of the array of claim 1. See Appendix A "Clean Version" and Appendix B "Version with Markings to Show Changes Made." Applicants believe this reply is fully responsive to this paragraph.

Conclusion

Examiner has provided information concerning communication and/or inquiries concerning this case. Applicants appreciate Examiner's willingness to communicate and assistance regarding this case. Applicants believe no response to this paragraph is necessary.

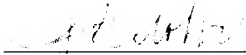
In view of the foregoing, and in summary, Applicants believe that all issues and points of the Examiner's Office action have been addressed, and that the amended claims (1-16) are patentable. Applicant respectfully requests reconsideration and allowance of this Application. Applicant

believes this paper to be fully responsive to each of the points made by the Examiner in the Action.

Please debit Deposit Account No. 50-0581 for any other deficiencies if necessary.

Dated this 15th day of July 2002.

Respectfully submitted,


Alison B. Mohr (Reg. No. 48,170)
PARSONS BEHLE & LATIMER
One Utah Center
201 South Main Street, Suite 1800
Post Office Box 45898
Salt Lake City, Utah 84145-0898
(801) 532-1234

Attorney Docket:
04537.002 / 3101

VERSION WITH MARKINGS TO SHOW CHANGES MADE**METHODS OF GENETIC ANALYSIS USING NUCLEIC ACID ARRAYS****CROSS REFERENCE TO RELATED APPLICATIONS**

The present application is a non-provisional application claiming priority from Provisional U.S. Patent Application Serial No. 60/100,678 filed September 17, 1998.

REFERENCE TO SEQUENCE LISTING

The sequence listing, including SEQ ID NOS: 1 - 127811, is contained on compact disc in two copies, labeled Copy 1 and Copy 2. The computer readable form is on a compact disc labeled CRF. The file name on each of the three compact discs is seqlist.rtf, created July 12, 2002. Each file is approximately 16.3 kilobytes. The sequence listing information recorded in the computer readable form is identical to the written compact disc sequence listing. The sequence listing is hereby incorporated in this application in its entirety and is to be considered part of the disclosure of this specification.

BACKGROUND OF THE INVENTION

The present invention provides a unique pool of nucleic acid sequences useful for analyzing molecular interactions of biological interest. The invention therefore relates to diverse fields impacted by the nature of molecular interaction, including chemistry, biology, medicine, and medical diagnostics.

FIELD OF THE INVENTION

Many biological functions are carried out by regulating the expression levels of various genes, either through changes in levels of transcription (*e.g.* through control of initiation, provision of RNA precursors, RNA processing, *etc.*) of particular genes, through changes in the

copy number of the genetic DNA, or through changes in protein synthesis. For example, control of the cell cycle and cell differentiation, as well as diseases, are characterized by the variations in the transcription levels of a group of genes.

Gene expression is not only responsible for physiological functions, but also associated with pathogenesis. For example, the lack of sufficient functional tumor suppressor genes and/or the over expression of oncogene/protooncogenes leads to tumorigenesis. (See, e.g., Marshall, Cell, 64: 313-326 (1991) and Weinberg, Science, 254: 1138-1146 (1991.)) Thus, changes in the expression levels of particular genes (e.g. oncogenes or tumor suppressors), serve as signposts for the presence and progression of various diseases.

As a consequence, novel techniques and apparatus are needed to study gene expression in specific biological systems.

All documents, i.e., publications and patent applications, cited in this disclosure, including the foregoing, are incorporated by reference herein in their entireties for all purposes to the same extent as if each of the individual documents were specifically and individually indicated to be so incorporated by reference herein in its entirety.

SUMMARY OF THE INVENTION

The invention provides nucleic acid sequences which are complementary to particular genes and makes them available for a variety of analyses, including, for example, gene expression analysis. For example, in one embodiment the invention comprises an array comprising of any 10 or more, 100 or more, 1000, or more, 10,000 or more or 100,000 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1 -127811, or the perfect match, perfect mismatch, antisense match or antisense mismatch thereof. In a further embodiment, the invention comprises the use of any of the above arrays or fragments disclosed in [TABLE 4]SEQ ID NOS: 1-127811 to: monitor gene expression

levels by hybridization of the array to a DNA library; monitor gene expression levels by hybridization to an mRNA protein fusion compound; identify polymorphisms; identify biallelic markers; produce genetic maps; analyze genetic variation; comparatively analyze gene expression between different species; analyze gene knockouts; or, to hybridize tag-labeled compounds. In a further embodiment the invention comprises a method of analysis comprising of hybridizing one or more pools of nucleic acids to two or more of the fragments disclosed in TABLE I and detecting said hybridization. In a further embodiment the invention comprises the use of any one or more of the fragments disclosed in [TABLE I]SEQ ID NOS: 1-127811 as a primer for PCR. In a further embodiment the invention comprises the use of any one or more of the fragments disclosed in [TABLE I]SEQ ID NOS: 1-127811 as a ligand.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Massive Parallel Screening: The phrase “massively parallel screening” refers to the simultaneous screening of at least about 100, preferably about 1000, more preferably about 10,000 and more preferably about 100,000 different nucleic acid hybridizations.

Nucleic Acid: The terms “nucleic acid” or “nucleic acid molecule” refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or double-stranded form, and unless otherwise limited, would encompass analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. Nucleic acids may be derived from a variety or sources including, but not limited to, naturally occurring nucleic acids, clones, synthesis in solution or solid phase synthesis.

Probe: As used herein a “probe” is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein,

a probe may include natural (*i.e.* A, G, U, C, or T) or modified bases (7-deazaguanosine, inosine, *etc.*). In addition, the bases in probes may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

Target nucleic acid: The term “target nucleic acid” or “target sequence” refers to a nucleic acid or nucleic acid sequence which is to be analyzed. A target can be a nucleic acid to which a probe will hybridize. The probe may or may not be specifically designed to hybridize to the target. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The term target nucleic acid may refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the overall sequence (*e.g.*, gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

mRNA or transcript: The term “mRNA” refers to transcripts of a gene. Transcripts are RNA including, for example, mature messenger RNA ready for translation, products of various stages of transcript processing. Transcript processing may include splicing, editing and degradation.

Subsequence: “Subsequence” refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

Perfect match: The term “match,” “perfect match,” “perfect match probe” or “perfect match control” refers to a nucleic acid that has a sequence that is perfectly complementary to a particular target sequence. The nucleic acid is typically perfectly complementary to a portion (subsequence) of the target sequence. A perfect match (PM) probe can be a “test probe”, a

“normalization control” probe, an expression level control probe and the like. A perfect match control or perfect match is, however, distinguished from a “mismatch” or “mismatch probe.”

Mismatch: The term “mismatch,” “mismatch control” or “mismatch probe” refers to a nucleic acid whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence. As a non-limiting example, for each mismatch (MM) control in a high-density probe array there typically exists a corresponding perfect match (PM) probe that is perfectly complementary to the same particular target sequence. The mismatch may comprise one or more bases. While the mismatch(es) may be located anywhere in the mismatch probe, terminal mismatches are less desirable because a terminal mismatch is less likely to prevent hybridization of the target sequence. In a particularly preferred embodiment, the mismatch is located at or near the center of the probe such that the mismatch is most likely to destabilize the duplex with the target sequence under the test hybridization conditions. A homo-mismatch substitutes an adenine (A) for a thymine (T) and vice versa and a guanine (G) for a cytosine (C) and vice versa. For example, if the target sequence was: AGGTCCA, a probe designed with a single homo-mismatch at the central, or fourth position, would result in the following sequence: TCCTGGT.

Array: An “array” is a solid support with at least a first surface having a plurality of different nucleic acid sequences attached to the first surface.

Gene Knockout: the term “gene knockout,” as defined in Lodish et al. *Molecular Cell Biology 3rd Edition*, Scientific American Books pub., which is hereby incorporated in its entirety for all purposes is, is a technique for selectively inactivating a gene by replacing it with a mutant allele in an otherwise normal organism.

DNA Library - as used herein the term “genomic library” or “genomic DNA library” refers to a collection of cloned DNA molecules consisting of fragments of the entire genome

(genomic library) or of DNA copies of all the mRNA produced by a cell type (cDNA library) inserted into a suitable cloning vector.

Polymorphism - "polymorphism" refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. A polymorphic marker or site is the locus at which divergence occurs. Preferred markers have at least two alleles, each occurring at a frequency of greater than 1%, and more preferably greater than 10% or 20% of the selected population. A polymorphic locus may be as small as one base pair. Polymorphic markers include restriction fragment length polymorphisms, variable number or tandem repeats (VNTR's), hypervariable regions, minisatellites, dinucleotide repeats, trinucleotide repeats, tetranucleotide repeats, simple sequence repeats, and insertion elements such as ALU. The first identified allelic form is arbitrarily designated as the reference form and other allelic forms are designated as alternative or variant alleles. The allelic form occurring most frequently in a selected population is sometimes referred to as the wildtype form. Diploid organisms may be homozygous or heterozygous for allelic forms. A diallelic or biallelic polymorphism has two forms. A triallelic polymorphism has three forms.

Genetic map - a "genetic map" is a map which presents the order of specific sequences on a chromosome.

Genetic variation - "genetic variation" refers to variation in the sequence of the same region between two or more organisms.

Hybridization - the association of two complementary nucleic acid strands or their derivatives (such as PNA) to form double stranded molecules. Hybrids can contain two DNA strands, two RNA strands, or one DNA and one RNA strand.

mRNA-protein fusion - a compound whereby an mRNA is directly attached to the peptide or protein it encodes by a stable covalent linkage.

Ligand - any molecule, other than an enzyme substrate, that binds tightly and specifically to a macromolecule, for example, a protein, forming a macromolecule-ligand complex.

II. General

[TABLE 4]SEQ ID NOS: 1-127811, encompassed in Appendix I, presents target sequences included in the invention. Each target sequence from columns 2,5 and 8 corresponds to and represents at least four additional nucleic acid sequences included in the invention. For example, if the first

nucleic acid sequence listed in [TABLE 4]SEQ ID NOS: 1-127811 is: 5'-cgccgaaagcgtccaatcaacat-3' the additional sequences included in the invention which are represented by this nucleic acid sequence are, for example:

[ggggecttgeaeggecagttgta]-3'-gcggccttcgcacggttagttgta-5' = (perfect) sense match

[ggggecttgec~~te~~gggecagttgta]-3'-gcggccttcgctcggttagttgta-5' = sense mismatch

[tacaactggcggagcgaaaggcege]-3'-tacaactaaccgagcgaaaggccgc-5' = [(~~perfect~~)]antisense mismatch

[tacaactggc~~gt~~ggcgaaggcege]-3'-tacaactaaccgtgcgaaggccgc-5' = [(perfect)]antisense mismatch

Accordingly, for each nucleic acid sequence listed in [TABLE 4]SEQ ID NOS: 1-127811, this disclosure includes the corresponding sense match, sense mismatch, antisense match and antisense mismatch. The position of the mismatch is not limited to the above example, it may be located anywhere in the nucleic acid sequence and may comprise one or more bases.

Consequently, the present invention includes: a) the target sequences listed in [TABLE 4]SEQ ID NOS: 1-127811, columns 2, 5 and 8 or the sense match, sense mismatch, antisense

match or antisense mismatch thereof; b) clones which comprise the target nucleic acid sequences listed in ~~[TABLE 4]~~SEQ ID NOS: 1-127811, columns 2, 5 and 8 or the sense-match, sense mismatch, antisense match or antisense mismatch thereof; c) longer nucleotide sequences which include the nucleic acid sequences listed in ~~[TABLE 4]~~SEQ ID NOS: 1-127811, columns 2, 5, and 8 or the sense match, sense mismatch, antisense match or antisense mismatch thereof and d) subsequences greater than 9 nucleotides in length of the target nucleic acid sequences listed in ~~[TABLE 4]~~SEQ ID NOS: 1-127811, columns 2, 5, and 8 or the sense match, sense mismatch, antisense match or antisense mismatch.

Target sequences were chosen from clusters of known murine genes available on the Unigene database as of August 15, 1996. Target sequences were selected using the computer based methods described in US P[~~p~~]atent ~~[application]~~No. 6,309,822 (issued October 30, 2001),[08/772,376] incorporated herein by reference for all purposes.

For each target sequence listed in ~~[TABLE 4]~~SEQ ID NOS: 1-127811 is a corresponding G[g]enbank database accession number. These accession numbers allow for the identification of sequences located in the G[g]enbank sequence database through the use of computer programs such as BLAST. Access to BLAST is available to the public through the Internet at, for example, <http://www.ncbi.nlm.nih.gov>. One of skill in the art will be familiar with the use of the BLAST program to obtain information about particular sequences in order to, for example, determine the species from which the sequence is derived, determine the gene from which the sequence is derived, to determine other genes and species which contain similar sequences and to determine the degree of similarity between one sequence and another. All information relating to the target sequences available through the G[g]enbank database is hereby incorporated by reference for all purposes.

The present invention provides a pool of unique nucleotide sequences complementary to murine genes and ESTs in particular embodiments which alone, or in combinations of 2 or more, 10 or more, 100 or more, 1,000 or more, 10,000 or more, or 100,000 or more, can be used for a variety of applications.

In one embodiment, the present invention provides for a pool of unique nucleotide sequences which are complementary to approximately 6500 murine genes formed into a high density array of probes suitable for array based massive parallel gene expression. Array based methods for monitoring gene expression are disclosed and discussed in detail in U.S. Patent Nos. [Applications,] 5,800,992 (issued September 9, 1998)[08/670,118], and 6,309,822 (issued October 30, 2001)[08/772,376] and PCT Application WO 92/10588 (published on June 25, 1992), all of which are incorporated herein by reference for all purposes. Generally those methods of monitoring gene expression involve (1) providing a pool of target nucleic acids comprising RNA transcript(s) of one or more target gene(s), or nucleic acids derived from the RNA transcript(s); (2) hybridizing the nucleic acid sample to a high density array of probes and (3) detecting the hybridized nucleic acids and calculating a relative expression (transcription, RNA processing or degradation) level.

The development of Very Large Scale Immobilized Polymer Synthesis or VLSIPS™ technology has provided methods for making very large arrays of nucleic acid probes in very small arrays. See U.S. Patent No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092, and Fodor *et al.*, *Science*, 251, 767-77 (1991), each of which is incorporated herein by reference. U.S. Patent application Serial No. 08/670,118, describes methods for making arrays of nucleic acid probes that can be used to detect the presence of a nucleic acid containing a specific nucleotide sequence. Methods of forming high density arrays of nucleic acids, peptides and other

polymer sequences with a minimal number of synthetic steps are known. The nucleic acid array can be synthesized on a solid substrate by a variety of methods, including, but not limited to, light-directed chemical coupling, and mechanically directed coupling.

In a preferred detection method, the array of immobilized nucleic acids, or probes, is contacted with a sample containing target nucleic acids, to which a fluorescent label is attached. Target nucleic acids hybridize to the probes on the array and any non-hybridized nucleic acids are removed. The array containing the hybridized target nucleic acids are exposed to light which excites the fluorescent label. The resulting fluorescent intensity, or brightness, is detected. Relative brightness is used to determine which probe is the best candidate for the perfect match to the hybridized target nucleic acid because fluorescent intensity (brightness) corresponds to binding affinity. Once the position of the perfect match probe is known, the sequence of the hybridized target nucleic acid is known because the sequence and position of the probe is known.

In the array of the present invention the probes are presented in pairs, one probe in each pair being a perfect match to the target sequence and the other probe being identical to the perfect match probe except that the central base is a homo-mismatch. Mismatch probes provide a control for non-specific binding or cross-hybridization to a nucleic acid in the sample other than the target to which the probe is directed. Thus, mismatch probes indicate whether a hybridization is or is not specific. For example, if the target is present, the perfect match probes should be consistently brighter than the mismatch probes because fluorescence intensity, or brightness, corresponds to binding affinity. (See, for example US Patent No. 5,324,633, which is incorporated herein for all purposes.) In addition, if all central mismatches are present, the mismatch probes can be used to detect a mutation. Finally the difference in intensity between the perfect match and the mismatch probe ($I(\text{PM}) - I(\text{MM})$) provides a good measure of the

concentration of the hybridized material. See pending PCT application No. 98/11223, which is incorporated herein by reference for all purposes. The probe pairs are presented in both sense and antisense orientation, thereby eliciting a total of four probes per target sequence: sense match, sense mismatch, antisense match and antisense mismatch.

In another embodiment, the current invention provides a pool of sequences which may be used as probes for their complementary genes listed in the G[g]enbank database. Methods for making probes are well known. See for example Sambrook, Fritsche and Maniatis. "Molecular Cloning A laboratory Manual" 2nd Ed. Cold Spring Harbor Press (1989) ("Maniatis et al.") which is hereby incorporated in its entirety by reference for all purposes. Maniatis et al. describes a number of uses for nucleic acid probes of defined sequence. Some of the uses described by Maniatis et al. include: to screen cDNA or genomic DNA libraries, or subclones derived from them, for additional clones containing segments of DNA that have been isolated and previously sequenced; in Southern, northern, or dot-blot hybridization to identify or detect the sequences of specific genes; in Southern, or dot-blot hybridization of genomic DNA to detect specific mutations in genes of known sequence; to detect specific mutations generated by site-directed mutagenesis of cloned genes; and to map the 5' termini of mRNA molecules by primer extensions. Maniatis et al. describes other uses for probes throughout. See also Alberts et al. *Molecular Biology of the Cell 3rd edition*, Garland Publishing Inc. (1994) p. 307 and Lodish et al. *Molecular Cell Biology, 3rd edition*, Scientific American Books (1995) p. 285-286, each of which is hereby incorporated by reference in its entirety for all purposes, for a brief discussion of the use of nucleic acid probes in *in situ hybridization*. Other uses for probes derived from the sequences disclosed in this invention will be readily apparent to those of skill in the art. See, for

example, Lodish et al. *Molecular Cell Biology*, 3rd edition, Scientific American Books (1995) p.229-233, incorporated above, for a description of the construction of genomic libraries.

In another embodiment, the current invention may be combined with known methods to monitor expression levels of genes in a wide variety of contexts. For example, where the effects of a drug on gene expression are to be determined, the drug will be administered to an organism, a tissue sample, or a cell and the gene expression levels will be analyzed. For example, nucleic acids are isolated from the treated tissue sample, cell, or a biological sample from the organism and from an untreated organism tissue sample or cell, hybridized to a high density probe array containing probes directed to the gene of interest and the expression levels of that gene are determined. The types of drugs that may be used in these types of experiments include, but are not limited to, antibiotics, antivirals, narcotics, anti-cancer drugs, tumor suppressing drugs, and any chemical composition which may affect the expression of genes *in vivo* or *in vitro*. The current invention is particularly suited to be used in the types of analyses described by, for example, pending US Patent[Applications] No. 6,309,822[~~08/772,376~~] and PCT Application No. 98/11223, each of which is incorporated by reference in its entirety for all purposes. As described in Wodicka et al., *Nature Biotechnology* 15 (1997), (hereby incorporated by reference in its entirety for all purposes), because mRNA hybridization correlates to gene expression level, hybridization patterns can be compared to determine differential gene expression. As non-limiting examples: hybridization patterns from samples treated with certain types of drugs may be compared to hybridization patterns from samples which have not been treated or which have been treated with a different drug; hybridization patterns for samples infected with a specific virus may be compared against hybridization patterns from non-infected samples; hybridization patterns for samples with cancer may be compared against hybridization patterns for samples

without cancer; hybridization patterns of samples from cancerous cells which have been treated with a tumor suppressing drug may be compared against untreated cancerous cells, etc. Zhang et al., Science 276 1268-1272, (hereby incorporated by reference in its entirety for all purposes), provides an example of how gene expression data can provide a great deal of insight into cancer research. One skilled in the art will appreciate that a wide range of applications will be available using 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more or 100,000 or more of the [Table 4]SEQ ID NOS: 1-127811 sequences as probes for gene expression analysis. The combination of the DNA array technology and the mouse specific probes in this disclosure is a powerful tool for studying gene expression.

In another embodiment, the invention may be used in conjunction with the techniques which link specific proteins to the mRNA which encodes the protein. (See for example Roberts and Szostak Proc. Natl. Acad. Sci. 94 12297-12302 (1997) which is incorporated herein in its entirety for all purposes.) Hybridization of these mRNA-protein fusion compounds to arrays comprised of 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more the sequences disclosed in the present invention provides a powerful tool for monitoring expression levels.

In one embodiment, the current invention provides a pool of unique nucleic acid sequences which can be used for parallel analysis of gene expression under selective conditions. Without wishing to be limited, genetic selection under selective conditions could include: variation in the temperature of the organism's environment; variation in pH levels in the organism's environment; variation in an organism's food (type, texture, amount etc.); variation in an organism's surroundings; etc. Arrays, such as those in the present invention, can be used to

determine whether gene expression is altered when an organism is exposed to selective conditions.

Methods for using nucleic acid arrays to analyze genetic selections under selective conditions are known. (See for example, R. Cho et al., Proc. Natl. Acad. Sci. 95 3752-3757 (1998) incorporated herein in its entirety for all purposes.) Cho et al. describes the use of a high-density array containing oligonucleotides complementary to every gene in the yeast *Saccharomyces cerevisiae* to perform two-hybrid protein-protein interaction screens for *S. cerevisiae* genes implicated in mRNA splicing and microtubule assembly. Cho et al. was able to characterize the results of a screen in a single experiment by hybridization of labeled DNA derived from positive clones. Briefly, as described by Cho et al., two proteins are expressed in yeast as fusions to either the DNA-binding domain or the activation domain of a transcription factor. Physical interaction of the two proteins reconstitutes transcriptional activity, turning on a gene essential for survival under selective conditions. In screening for novel protein-protein interactions, yeast cells are first transformed with a plasmid encoding a specific DNA-binding fusion protein. A plasmid library of activation domain fusions derived from genomic DNA is then introduced into these cells. Transcriptional activation fusions found in cells that survive selective conditions are considered to encode peptide domains that may interact with the DNA binding domain fusion protein. Clones are then isolated from the two-hybrid screen and mixed into a single pool. Plasmid DNA is purified from the pooled clones and the gene inserts are amplified using PCR. The DNA products are then hybridized to yeast whole genome arrays for characterization. The methods employed by Cho et al. are applicable to the analysis of a range of genetic selections. High density arrays created using two or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of the sequences disclosed in the current invention

can be used to analyze genetic selections in the mouse system using the methods described in Cho et al.

In another embodiment, the current invention provides a pool of unique nucleic acid sequences which can be used to identify biallelic markers, providing a novel and efficient approach to the study of genetic variation. For example, methods for using high density arrays comprised of probes which are complementary to the genomic DNA of a particular species to interrogate polymorphisms are well known. (See for example, US Patent No. 6,300,063 (issued October 9, 2001) and US Patent Application No[s]. 08/965,620 [and 08/853,370] which are hereby incorporated herein for all purposes.) Pools of 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of the sequences disclosed in this invention combined with the methods described in the above patent applications provides a tool for studying genetic variation in the mouse system.

In another embodiment of the invention, genetic variation can be used to produce genetic maps of various strains of mouse. Winzler et al., "Direct Allelic Variation Scanning of the Yeast Genome" Science (in press) (1998), which is hereby incorporated for all purposes describe methods for conducting this type of screening with arrays containing probes complementary to the yeast genome. Briefly, genomic DNA from strains which are phenotypically different are isolated, fragmented, and labelled. Each strain is then hybridized to identical arrays comprised of the nucleic acid sequences complementary to the system being studied. Comparison of hybridization patterns between the various strains then serve as genetic markers. As described by Winzler et al, these markers can then be used for linkage analysis. High density arrays created from 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of

the sequences disclosed in this invention can be used to study genetic variation using the methods described by Winzler et al.

In another embodiment, the present invention may be used for cross-species comparisons. One skilled in the art will appreciate that it is often useful to determine whether a gene present in one species, for example the mouse, is present in a conserved format in another species, including, without limitation, mouse, human, chicken, zebrafish, *drosophila*, or yeast. See, for example, Andersson et al., Mamm Genome 7(10):717-734 (1996,) which is hereby incorporated by reference for all purposes, which describes the utility of cross-species comparisons. The use of 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more or 100,000 or more of the sequences disclosed in this invention in an array can be used to determine whether any of the sequence from one or more of the murine genes represented by the sequences disclosed in this invention is conserved in another species by, for example, hybridizing genomic nucleic acid samples from another species to an array comprised of the sequences disclosed in this invention. Areas of hybridization will yield genomic regions where the nucleotide sequence is highly conserved between the interrogation species and the mouse.

In another embodiment, the present invention may be used to characterize the genotype of knockouts. Methods for using gene knockouts to identify a gene are well known. See for example, Lodish et al. *Molecular Cell Biology, 3rd Edition*, Scientific American Books pub pp. 292-296 and US Patent No. 5,679,523 which are hereby incorporated by reference for all purposes. By isolating genomic nucleic acid samples from knockout species with a known phenotype and hybridizing the samples to an array comprised of 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of the sequences disclosed in this

invention, candidate genes which contribute to the phenotype will be identified and made accessible for further characterization.

In another embodiment, the present invention may be used to identify new gene family members. Methods of screening libraries with probes are well known. (See, for example, Maniatis et al, incorporated by reference above.) Because the present invention is comprised of nucleic acid sequences from specific known genes, 2 or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of sequences disclosed in this invention may be used as probes to screen genomic libraries to look for additional family members of those genes from which the target sequences are derived.

In another embodiment, the present invention may be used to provide nucleic acid sequences to be used as tag sequences. Tag sequences are a type of genetic "bar code" which can be used to label compounds of interest. The analysis of deletion mutants using tag sequences is described in, for example, Shoemaker et al., Nature Genetics 14 450-456 (1996), which is hereby incorporated by reference in its entirety for all purposes. Shoemaker et al. describes the use of PCR to generate large numbers of deletion strains. Each deletion strain is labelled with a unique 20-base tag sequence that can be hybridized to a high-density oligonucleotide array. The tags serve as unique identifiers (molecular bar codes) that allow analysis of large numbers of deletion strains simultaneously through selective growth conditions. The use of tag sequences need not be limited to this example however. The utility of using unique known short oligonucleotide sequences capable of hybridizing to a nucleic acid array to label various compounds will be apparent to one skilled in the art. One or more, 10 or more, 100 or more, 1000 or more, 10,000 or more, or 100,000 or more of the [Table 4]SEQ ID NOS: 1-127811 sequences are excellent candidates to be used as tag sequences.

In another embodiment of the invention, the sequences of this invention may be used to generate primers directed to their corresponding genes as disclosed in the Genbank or any other public database. These primers may be used in such basic techniques as sequencing or PCR, see for example Maniatis et al., incorporated by reference above.

In another embodiment, the invention provides a pool of nucleic acid sequences to be used as ligands for specific genes. The sequences disclosed in this invention may be used as ligands to their corresponding genes as disclosed in the Genbank or any other public database. Compounds which specifically bind known genes are of interest for a variety of uses. One particular clinical use is to act as an antisense protein which specifically binds and disables a gene which has been, for example, linked to a disease. Methods and uses for ligands to specific genes are known. See for example, US Patent No. 5,723,594 which is hereby incorporated by reference in its entirety for all purposes.

In a preferred embodiment, the hybridized nucleic acids are detected by detecting one or more labels attached to the sample nucleic acids. The labels may be incorporated by any of a number of means well known to those of skill in the art. In one embodiment, the label is simultaneously incorporated during the amplification step in the preparation of the sample nucleic acids. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In a another embodiment, transcription amplification, as described above, using a labeled nucleotide (*e.g.* fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acids.

Alternatively, a label may be added directly to the original nucleic acid sample (*e.g.*, mRNA, polyA mRNA, cDNA, *etc.*) or to the amplification product after the amplification is completed. Means of attaching labels to nucleic acids are well known to those of skill in the art

and include, for example nick translation or end-labeling (*e.g.* with a labeled RNA) by kinasing of the nucleic acid and subsequent attachment (ligation) of a nucleic acid linker joining the sample nucleic acid to a label (*e.g.*, a fluorophore).

Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (*e.g.*, DynabeadsTM), fluorescent dyes (*e.g.*, fluorescein, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (*e.g.*, ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), phosphorescent labels, enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each of which is hereby incorporated by reference in its entirety for all purposes.

Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

The label may be added to the target nucleic acid(s) prior to, or after the hybridization. So called "direct labels" are detectable labels that are directly attached to or incorporated into the target nucleic acid prior to hybridization. In contrast, so called "indirect labels" are joined to the

hybrid duplex after hybridization. Often, the indirect label is attached to a binding moiety that has been attached to the target nucleic acid prior to the hybridization. Thus, for example, the target nucleic acid may be biotinylated before the hybridization. After hybridization, an avidin-conjugated fluorophore will bind the biotin bearing hybrid duplexes providing a label that is easily detected. For a detailed review of methods of labeling nucleic acids and detecting labeled hybridized nucleic acids see *Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization With Nucleic Acid Probes*, P. Tijssen, ed. Elsevier, N.Y., (1993) which is hereby incorporated by reference in its entirety for all purposes.

Fluorescent labels are preferred and easily added during an *in vitro* transcription reaction. In a preferred embodiment, fluorescein labeled UTP and CTP are incorporated into the RNA produced in an *in vitro* transcription reaction as described above.

EXAMPLE

The following example serves to illustrate the type of experiment that could be conducted using the invention.

Expression Monitoring by Hybridization to High Density Oligonucleotide Arrays Arrays containing the desired number of probes can be synthesized using the method described in US Patent No. 5,143,854, incorporated by reference above. Extracted poly (A)⁺RNA can then be converted to cDNA using the methods described below. The cDNA is then transcribed in the presence of labeled ribonucleotide triphosphates. The label may be biotin or a dye such as fluorescein. RNA is then fragmented with heat in the presence of magnesium ions. Hybridizations are carried out in a flow cell that contains the two-dimensional DNA probe arrays. Following a brief washing step to remove unhybridized RNA, the arrays are scanned using a scanning confocal microscope.

1. A method of RNA preparation:

Labeled RNA is prepared from clones containing a T7 RNA polymerase promoter site by incorporating labeled nucleotides in an IVT reaction. Either biotin-labeled or fluorescein-labeled UTP and CTP (1:3 labeled to unlabeled) plus unlabeled ATP and GTP is used for the reaction with 2500 U of T7 RNA polymerase. Following the reaction unincorporated nucleotide triphosphates are removed using size-selective membrane such as Microcon - 100, (Amicon, Beverly, MA). The total molar concentration of RNA is based on a measurement of the absorbance at 260 nm. Following quantitation of RNA amounts, RNA is fragmented randomly to an average length of approximately 50 bases by heating at 94° in 40 mM Tris-acetate pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate, for 30 to 40 min. Fragmentation reduces possible interference from RNA secondary structure, and minimizes the effects of multiple interactions with closely spaced probe molecules. For material made directly from cellular RNA, cytoplasmic RNA is extracted from cells by the method of Favaloro et al. *Methods Enzymol.* 65:718-749 (1980) hereby incorporated by reference for all purposes, and poly (A)⁺ RNA is isolated with an oligo dT selection step using, for example, Poly Atract, (Promega, Madison, WI). RNA can be amplified using a modification of the procedure described by Eberwine et al. *Proc. Natl. Acad. Sci. USA* 89:3010-3014 (1992) hereby incorporated by reference for all purposes. Microgram amounts of poly (A)⁺ RNA are converted into double stranded cDNA using a cDNA synthesis kit (kits may be obtained from Life Technologies, Gaithersburg, MD) with an oligo dT primer incorporating a T7 RNA polymerase promoter site. After second-strand synthesis, the reaction mixture is extracted with phenol/chloroform, and the double-stranded DNA isolated using a membrane filtration step using, for example, Microcon -100, (Amicon). Labeled cRNA can be made directly from the cDNA pool with an IVT step as described above.

The total molar concentration of labeled cRNA is determined from the absorbance at 260nm and assuming an average RNA size of 1000 ribonucleotides. The commonly used convention is that 1 OD is equivalent to 40 ug of RNA, and that 1 ug of cellular mRNA consists of 3 pmol of RNA molecules. Cellular mRNA may also be labeled directly without any intermediate cDNA synthesis steps. In this case, Poly (A)⁺ RNA is fragmented as described, and the 5' ends of the fragments are kinased and then incubated overnight with a biotinylated oligoribonucleotide (5'-biotin-AAAAAA-3') in the presence of T4 RNA ligase (available from Epicentre Technologies, Madison, WI). Alternatively, mRNA has been labeled directly by UV-induced cross-linking to a psoralen derivative linked to biotin (available from Schleicher & Schuell, Keene, NH).

2. Array hybridization and Scanning:

Array hybridization solutions can be made containing 0.9 M NaCl, 60mM EDTA, and 0.005% of the product octylphenol ethylene oxide condensate sold under the trademark Triton® as described by Sigma Product number X-100~~[Triton X-100]~~, adjusted to pH 7.6 (referred to as 6xSSPE-T). In addition, the solutions should contain 0.5 mg/ml unlabeled, degraded herring sperm DNA (available from Sigma, St. Louis, MO). Prior to hybridization, RNA samples are heated in the hybridization solution to 99°C for 10 min, placed on ice for 5 min, and allowed to equilibrate at room temperature before being placed in the hybridization flow cell. Following hybridization, the solutions are removed, the arrays washed with 6xSSPE-T at 22°C for 7 min, and then washed with 0.5xSSPE-T at 40°C for 15 min. When biotin labeled RNA is used the hybridized RNA should be stained with a streptavidin-phycoerythrin in 6xSSPE-T at 40°C for 5 min. The arrays are read using a scanning confocal microscope made by Molecular Dynamics (commercially available through Affymetrix, Santa Clara, CA). The scanner uses an argon ion laser as the excitation source, with the emission detected by a photomultiplier tube through either

a 530 nm bandpass filter (fluorescein) or a 560 nm longpass filter (phycoerythrin). Nucleic acids of either sense or antisense orientations may be used in hybridization experiments. Arrays for probes with either orientation (reverse complements of each other) are made using the same set of photolithographic masks by reversing the order of the photochemical steps and incorporating the complementary nucleotide.

3. Quantitative analysis of hybridization patterns and intensities.

Following a quantitative scan of an array, a grid is aligned to the image using the known dimensions of the array and the corner control regions as markers. The image is then reduced to a simple text file containing position and intensity information using software developed at Affymetrix (available with the confocal scanner). This information is merged with another text file that contains information relating physical position on the array to probe sequence and the identity of the RNA (and the specific part of the RNA) for which the oligonucleotide probe is designed. The quantitative analysis of the hybridization results involves a simple form of pattern recognition based on the assumption that, in the presence of a specific RNA, the perfect match (PM) probes will hybridize more strongly on average than their mismatch (MM) partners. The number of instances in which the PM hybridization is larger than the MM signal is computed along with the average of the logarithm of the PM/MM ratios for each probe set. These values are used to make a decision (using a predefined decision matrix) concerning the presence or absence of an RNA. To determine the quantitative RNA abundance, the average of the difference (PM-MM) for each probe family is calculated. The advantage of the difference method is that signals from random cross-hybridization contribute equally, on average, to the PM and MM probes, while specific hybridization contributes more to the PM probes. By averaging the

pairwise differences, the real signals add constructively while the contributions from cross-hybridization tend to cancel. When assessing the differences between two different

RNA samples, the hybridization signals from side-by-side experiments on identically synthesized arrays are compared directly. The magnitude of the changes in the average of the difference (PM-MM) values is interpreted by comparison with the results of spiking experiments as well as the signals observed for the internal standard bacterial and phage RNAs spiked into each sample at a known amount. Data analysis programs, such as those described in US Patent No. 08/828,952 perform these operations automatically.

CONCLUSION

The inventions herein provide a pool of unique nucleic acid sequences which are complementary to approximately 6500 specific known murine genes. These sequences can be used for a variety of types of analyses.

The above description is illustrative and not restrictive. Many variations of the invention will become apparent to those of skill in the art upon review of this disclosure. The scope of the invention should, therefore, be determined not with reference to the above description, but instead be determined with reference to the appended claims along with their full scope of equivalents.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

What is claimed is:

1. An array comprising of any 10 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1-127811, or the sense[~~perfect~~] match, sense[~~perfect~~] mismatch, antisense match or antisense mismatch thereof.
2. The array of claim 1 comprising any 100 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1-127811, or the sense[~~perfect~~] match, sense[~~perfect~~] mismatch, antisense match or antisense mismatch thereof.
3. The array of claim 1 comprising any 1000 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1-127811, or the sense[~~perfect~~] match, sense[~~perfect~~] mismatch, antisense match or antisense mismatch thereof.
4. The array of claim 1 comprising any 10,000 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1-127811, or the 15 sense[~~perfect~~] match, sense[~~perfect~~] mismatch, antisense match or antisense mismatch thereof.
5. The array of claim 1 comprising any 100,000 or more nucleic acid probes containing 9 or more consecutive nucleotides from the sequences listed in SEQ ID NOS: 1-127811, or the sense[~~perfect~~] match, sense[~~perfect~~] mismatch, antisense match or antisense mismatch thereof.
6. A method of using t[~~F~~]he array of claim 1 wherein said array is used to monitor gene expression levels.
7. A method of using t[~~F~~]he array of claim 1 wherein said array is used to monitor gene expression levels by hybridization to a DNA library.

8. A method of using t[\mp]he array of claim 1 wherein said array is used to monitor
gene expression levels by hybridization to an mRNA-protein fusion compound.

9. A method of using t[\mp]he array of claim 1 wherein said array is used for analysis
of genetic selections.

10. A method of using t[\mp]he array of claim 1 wherein said array is used for
identification of polymorphisms.

11. A method of using t[\mp]he array of claim 1 wherein said array is used for
identification of biallelic markers.

12. A method of using t[\mp]he array of claim 1 wherein said array is used for the
production of genetic maps.

13. A method of using t[\mp]he array of claim 1 wherein said array is used for analysis
of genetic variation.

14. A method of using t[\mp]he array of claim 1 wherein said array is used for
comparative analysis of gene expression in mouse and. another species.

15. A method of using t[\mp]he array of claim 1 wherein said array is used to analyze a
gene knockout.

16. A method of using t[\mp]he array of claim 1 wherein said array is used for
hybridization of tag-labeled compounds.

GeneChip[®] Murine Genome U74v2 Set

Extensive Coverage of the Mouse Genome

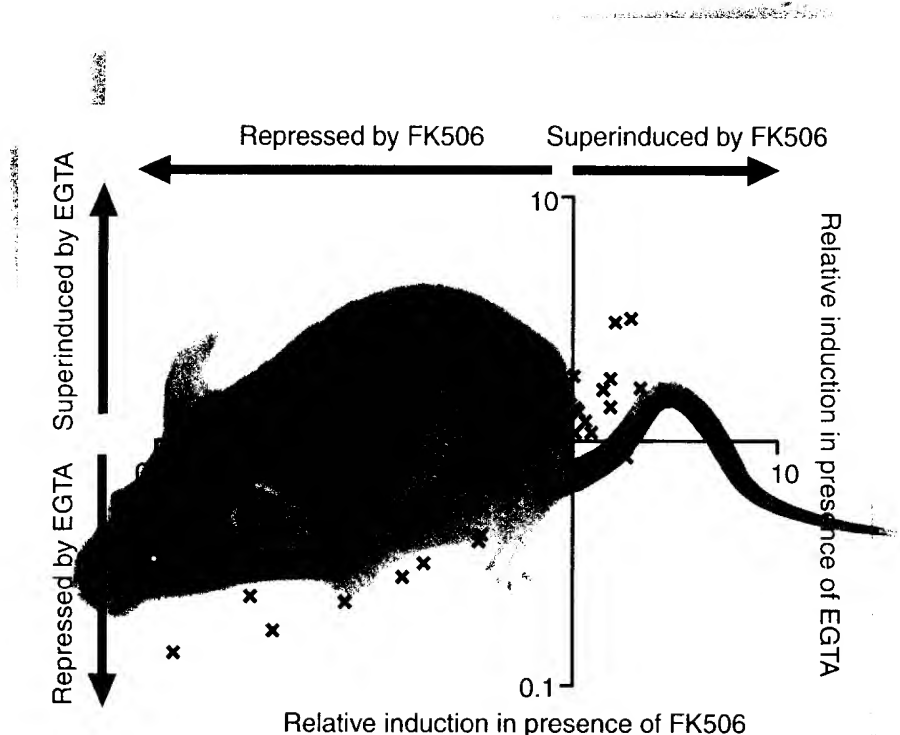
- Measure the temporal and spatial expression levels of more than 36,000 mouse genes and ESTs using the Murine Genome U74v2 Set. This set contains the most comprehensive transcript coverage of the mouse genome currently available.

Applications

The Murine Genome U74v2 Set is a powerful genetic tool developed to analyze mouse cells and tissues, the most widely used mammalian model in biomedical research.

Define Relationships Between Known Genes

The first array in the set, the Murine Genome U74Av2 Array (MG-U74Av2) represents all sequences (~6,000) in the Mouse UniGene database (Build 74) that have been functionally characterized. In addition, ~6,000 EST clusters are also represented on this single array. MG-U74Av2 can help you identify gene associations in a particular cell type or tissue, define key targets in signaling pathways and address many other interesting experimental questions.



Data from a study designed to understand how self-tolerance and the immunosuppressive drug FK506 prevent B-cell mitogenesis. This figure depicts data from mouse GeneChip[®] microarrays comparing genes that are regulated by the addition of either FK506 or EGTA during antigen-specific activation. The responses are virtually identical, indicating that the FK506 effect on gene expression is through a calcium-dependent pathway. Glynne et al, *Nature* **403**, 672-676 (2000). Reprinted by permission from *Nature*, copyright 2000. Macmillan Magazines Ltd.

Discover and Define Gene Function

In total, approximately 30,000 EST clusters are represented on the Murine Genome U74v2 Set. Use these arrays to discover gene function for undefined and uncharacterized gene sequences represented as expressed sequence tags.

Build Quantitative Databases

GeneChip[®] expression arrays allow for highly parallel, reproducible, quantification of gene expression levels. The Murine Genome U74v2 Set is an ideal tool for developing robust mouse expression databases to accelerate your research efforts.

AFFYMETRIX[®]

Data Sets Profiled

The Murine Genome U74v2 Set is comprised of three arrays and represents ~ 36,000 full-length genes and EST clusters. The sequences represented are derived from sequence clusters in Build 74 of the UniGene Database. UniGene clusters are represented by one or more consensus sequences derived directly from cluster members.

Relationship of Arrays in Set and Portfolio

The Murine Genome U74v2 Set is an update to the original Murine Genome U74 Set, and corrects non-functional probe sets on all three arrays.

The original Murine Genome U74 Set was designed to represent all sequences previously represented on the Mu11K Set and all of the UniGene sequences on the Mu19K Set.

All sequences previously represented by the Mu11K Set are represented on the MG-U74Av2 Array of the new set. The sequences from Mu19K Set are represented on both the A and B arrays.

Due to the dynamic nature of public databases, probe sets for these sequences will not be identical, and in some cases, will be represented by a completely new probe set. As a result, data generated with different versions of murine arrays may not produce concordant results. For more information, please contact Affymetrix technical support.

Specifications

Number of Arrays in Set	3
Array Size	Standard format
Feature Size	20 µm
Oligonucleotide Probe Length	25mer
Probe Pairs/Sequence	~ 16
Sensitivity	1:100,000
Control sequences	
Hybridization controls	<i>bioB</i> , <i>bioC</i> , <i>bioD</i> and <i>cre</i>
Poly A controls	<i>dap</i> , <i>lys</i> , <i>pbc</i> , <i>thr</i> , and <i>trp</i>
Maintenance Genes	actin, GAPDH, hexokinase

Ordering Information:

Part No.	Name	Description
900343	Murine Genome U74Av2	Contains 5 Arrays
900344	Murine Genome U74Av2	Contains 30 Arrays
900357	Murine Genome U74Bv2	Contains 5 Arrays
900358	Murine Genome U74Bv2	Contains 30 Arrays
900359	Murine Genome U74Cv2	Contains 5 Arrays
900360	Murine Genome U74Cv2	Contains 30 Arrays

AFFYMETRIX, INC.

3380 Central Expressway
Santa Clara, CA 95051 USA
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441
sales@affymetrix.com
support@affymetrix.com

AFFYMETRIX UK Ltd.,




Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom
Tel: +44 (0)1628 552550
Fax: +44 (0)1628 552585
saleseurope@affymetrix.com
supporteurope@affymetrix.com

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